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Exercise-Induced Changes in the Cortical Bone of Growing Mice Are Bone and Gender Specific

Joseph M. Wallace[1], **Rupak M. Rajachar**[1], **Matthew R. Allen**[4], **Susan A. Bloomfield**[4], **Pamela G. Robey**[3], **Marian F. Young**[3], and **David H. Kohn**[1,2,3,*]

Joseph M. Wallace: jmwallac@umich.edu; Rupak M. Rajachar: rupakr@u.washington.edu; Matthew R. Allen: matallen@iupui.edu; Susan A. Bloomfield: sbloom@hlkn.tamu.edu; Pamela G. Robey: probey@dir.nidcr.nih.gov; Marian F. Young: myoung@dir.nidcr.nih.gov; David H. Kohn: dhkohn@umich.edu

[1] Department of Biomedical Engineering, University of Michigan, Ann Arbor, MI

[2] Department of Biologic and Materials Sciences, University of Michigan, Ann Arbor, MI

[3] National Institutes of Health, National Institute of Dental and Craniofacial Research, Craniofacial and Skeletal Diseases Branch, Department of Health and Human Services; Bethesda, MD

[4] Texas A&M University, Department of Health and Kinesiology; College Station, TX

Abstract

Fracture risk and mechanical competence of bone are functions of bone mass and tissue quality, which in turn are dependent on the bone's mechanical environment. Male mice have a greater response to non weight-bearing exercise than females, resulting in larger, stronger bones compared with control animals. The aim of this study was to test the hypothesis that short-term weight-bearing running during growth (21 days starting at 8 weeks of age; 30 minutes/day; 12 meters/minute; 5° incline; 7 days/week) would similarly have a greater impact on cross sectional geometry and mechanical competence in the femora and tibiae of male mice versus females. Based on the orientation of the legs during running and the proximity of the tibia to the point of impact, this response was hypothesized to be greatest in the tibia. Exercise-related changes relative to controls were assayed by four-point bending tests, while volumetric bone mineral density and cross-sectional geometry were also assessed. The response to running was bone and gender-specific, with male tibiae demonstrating the greatest effects. In male tibiae, periosteal perimeter, endocortical perimeter, cortical area, medial-lateral width and bending moment of inertia increased versus control mice suggesting that while growth is occurring in these mice between 8 and 11 weeks of age, exercise accelerated this growth resulting in a greater increase in bone tissue over the 3 weeks of the study. Exercise increased tissue-level strain-to-failure and structural post-yield deformation in the male tibiae, but these post-yield benefits came at the expense of decreased yield deformation, structural and tissue-level yield strength and tissue-level ultimate strength. These results suggest that exercise superimposed upon growth accelerated growth-related increases in tibial cross-sectional dimensions. Exercise also influenced the quality of this forming bone, significantly impacting structural and tissue-level mechanical properties.

Corresponding Author: David H. Kohn, Ph.D., University of Michigan, Department of Biologic & Materials Sciences, 1011 N. University Ave., Ann Arbor, MI 48109-1078, Ph: (734) 764-2206, Fax: (734) 647-2110, E-mail: dhkohn@umich.edu.
Current Author Affiliations: Rupak M. Rajachar – Department of Biomedical Engineering, Michigan Technological University; Houghton, MI.
Matthew R. Allen – Department of Anatomy & Cell Biology, Indiana University School of Medicine; Indianapolis, IN.
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Keywords

mechanical properties; pQCT; matrix changes; tibia; femur

INTRODUCTION

Skeletal fragility leading to fracture is a significant medical and economic burden facing society. Each year, an estimated 1.5 million Americans suffer an age-related fracture, resulting in direct care expenditures of 18 billion dollars a year, measured in 2002 dollars [1]. Though skeletal fragility is often considered an age-related condition, stress and/or trauma-related fractures are a clinically significant problem in younger people as well [2,3]. The weight bearing bones of the lower extremities, particularly the cortical bone of the tibial and metatarseal diaphyses, are most often affected [4,5]. However, fracture risk is not equivalent between genders, with female long-distance runners and military recruits demonstrating a higher propensity for stress-fractures than males [6–8].

It is generally accepted that fracture risk and mechanical integrity of bone are not only affected by bone mass, but are also functions of tissue quality [9]. While influenced by many environmental factors (e.g. nutrition, hormones, pharmacological factors), the quantity and quality of bone are also significantly dependent on biomechanical influences. The importance of skeletal loading for the maintenance and increase of bone mass was suggested by Wolff as early as 1892 [10], and is universally accepted today.

Many animal models exist to study the effects of mechanical stimulation on bone structure and tissue quality, and ultimately skeletal function. Multiple exercise-based rat models have shown a link between the loading of bone and increased bone formation, resulting in increased bone mass and maintenance or improvement of mechanical properties [11–14]. Exercise also increases bone formation in mice, resulting in increased bone cross-sectional geometric properties and mass [15–19]. The primary deficiency in most murine exercise studies is that few investigate mechanical properties. The consensus from those murine studies that do look at mechanical properties is that moderate exercise improves or maintains whole-bone strength, though only a limited number of properties are reported in these studies (e.g. structural strength, ultimate stress, modulus, total deformation). Since the mechanical competence of bone is a clinically important metric, information regarding the alteration of a range mechanical properties following an exercise regimen, including changes in energy dissipation and deformation, is essential to assess the efficacy of the exercise and its ability to increase fracture resistance.

The bones of male mice show greater sensitivity to swimming than females [17,18]. However, weight-bearing exercise may be more efficient than non-weight-bearing exercise at eliciting structural changes in bone due to the direct loading of the whole bone rather than loading applied mainly at points of muscle insertion [20,21]. Further, the age at the initiation of such loading is important, since the growing skeleton has a greater capacity to accrue bone than does the adult skeleton [22–24]. Therefore, the aim of this study was to test the hypothesis that short-term weight-bearing exercise during growth would have a greater positive impact on cross sectional geometry and mechanical competence in the femora and tibiae of male C57BL6/129 mice versus the response in females. Further, based on the orientation of the legs during running and the proximity of the tibia to the point of impact, the response to this type of exercise was hypothesized to be greater in the tibia versus the femur. At 11 weeks of age and after 21 consecutive days of training, exercise-related changes relative to non-exercise controls were assessed in the cortical bone of the femora and tibiae of both genders. Four-point bending tests were performed to determine mechanical properties at the whole bone (structural)

and tissue levels. Additionally, volumetric bone mineral density (from peripheral quantitative computed tomography) and cross-sectional geometry were assessed.

MATERIALS AND METHODS

Animals and Treatment

All animal procedures were performed at the National Institute of Dental and Craniofacial Research (NIDCR) with NIDCR Institutional Animal Care and Use Committee approval (NIDCR animal approval protocol #NIDCR 001–151). To determine proper sample sizes for detecting effects of exercise across bones and gender, power calculations were performed based on published values for differences and standard deviations in mechanical and geometric properties due to mechanical loading in C57BL6 mice [19] using a value of α=0.05 and a power (1-β) of 0.80.

Mice were bred on a C57BL6/129 (B6;129) background [25]. Twenty mice from each gender (2 exercise groups, 10 mice per group) were housed in standard cages and given access to food, water and cage activity *ad libitum*. At 8 weeks of age, mice from each gender were randomly assigned to 1 of 2 weight matched groups (control, exercise). Exercise consisted of running on a treadmill (12 meters/minute at a 5° incline) for 30 minutes/day, 7 days/week for 21 consecutive days (Columbus Instruments, Model 1055M, Columbus, OH). Each lane of the treadmill was equipped with an adjustable-amperage (0–1.5 mA) shock grid at the rear of the belt to stimulate each mouse to run independently of all others. By the end of the second day of the experiment, all mice were running without the need of shock stimulation. Three days after the end of exercise, animals were sacrificed, at which time body weights were measured again and both femora and tibiae were harvested, stripped of soft tissue and stored in either 70% ethanol at 4° C (for peripheral quantitative computed tomography (pQCT)) or wrapped in gauze soaked in a Ca^{2+}-buffered saline solution at −80°C (for mechanical testing). The bones used for mechanical testing were brought to room temperature before testing.

Mechanical Testing

Left femora and tibiae were tested to failure in 4 point bending in displacement control using a custom-designed, solenoid driven loading apparatus with a support span (L) of 6 mm and a loading span of 4 mm at a rate of 0.01 mm/sec [25,26]. Before testing, the length of each femur was measured from the greater trochanter to the most distal portion of the femoral chondyles and the length of each tibia was measured from the most proximal portion of the tibial plateau to the most distal portion of the medial malleolus using digital calipers accurate to 0.01 mm (Mitutoyo; Aurora, IL). Femora were tested in the anterior-posterior (AP) direction (anterior surface in tension) with the middle of the bone positioned halfway between the two supports. Tibiae were tested in the medial-lateral (ML) direction (medial surface in tension). The tibiae were positioned such that the most distal portion of the junction of the tibia and fibula (TFJ) was aligned with the left-most support point. During each test, load and deflection were recorded, from which structural strength (yield force and ultimate force), energy or work (measured as the area under the force vs. displacement curve to yield and to failure), stiffness (the slope of the linear portion of the force vs. displacement curve) and deformation (yield deformation, total deformation and post-yield deformation) were derived at the whole bone level [25]. Load was measured using a linear force transducer with an operating range of 0–25 lb (Sensotec, Precision Linear Load Cell Model 34; Columbus, OH) while deflection was measured using an eddy current sensor with 0.27 μm resolution and operating range of 0–3.81 mm (Kaman Measuring Systems, Model 2440 5CM; Middletown, CT).

During each test, the bone was visually monitored and the point of fracture initiation was noted. Because fractures often propagated at an angle across the bone (i.e. oblique fractures), the half

of the fractured bone containing both the fracture initiation site and a full planar section of bone transverse to that site was dehydrated in graded ethanol (70%, 95%, 100%) at 4°C, defatted in acetone and infiltrated in a liquid methyl methacrylate monomer (Koldmount™ Cold Mounting Liquid; Mager Scientific) [25]. The bones were then embedded in methyl methacrylate (Koldmount™ Cold Mounting Kit; Mager Scientific). Using a low-speed sectioning saw (South Bay Technology, Model 650; San Clemente, CA) with a diamond wafering blade (Mager Scientific; Dexter, MI), the jagged edge of the bone adjacent to the fracture site was removed. A 200 μm thick planar section (hand ground to a final thickness of between 50 and 75 μm using wet silicon carbide abrasive discs) was then used to determine geometric parameters (average cortical thickness from four quadrants, cortical area, and AP and ML diameters) using a light microscope and digital analysis software (Nikon Eclipse TE 300, Image Pro-Plus v4.5, Matlab v5.3) [25]. The moment of inertia (I) about the axis of bending (I_{ML} for the femur and I_{AP} for the tibia) was measured from a binary image of each section using a Matlab script that integrates the distribution of material points about the calculated neutral axes [27]. Together with the load and deflection data, the distance from the centroid to the surface of the bone in tension (c) and I were used to map force and displacement (structural level) into stress and strain (tissue level) from standard beam-bending equations for 4 point bending:

$$Stress=\sigma=\frac{Fac}{2I}\ (MPa) \qquad Strain=\varepsilon=\frac{6cd}{a(3L-4a)}\ x\ 10^6\ (\mu\varepsilon)$$

In these equations, F is the force, d is the displacement, a is the distance from the support to the inner loading point (1 mm) and L is the span between the outer supports (6 mm). The yield point was calculated using the 0.2% offset method based on the stress-strain curve [28]. The modulus of elasticity was calculated as the slope of the linear portion of the stress-strain curve.

Peripheral Quantitative Computed Tomography (pQCT)

Ex vivo scans of right femora and tibiae from the male mice were performed at Texas A&M University using an XCT Research-M device (Stratec Corp., Norland, Fort Atkinson, WI) [29]. Machine calibration was performed using a hydroxyapatite cone phantom. Scans were performed at a scan speed of 2.5 mm/sec with a voxel resolution of 70 μm^2 and a scanning beam thickness of 0.50 mm. Two slices were scanned at the mid-diaphysis of each bone (50% of the total bone length ± 0.50 mm). A standardized analysis of diaphyseal bone (threshold of 700 mg/cm^3) was applied to each section. Values of total volumetric bone mineral density (vBMD, coefficient of variation = ±0.63 %) obtained for the two slices were averaged to get mean values for each bone. Because of the small size of bones from these mice (cortical thickness on the order of 200 μm) and the scanning resolution of 70 μm^2, there is an increased probability of error in clearly defining the edges of the bones [30,31]. Therefore, geometric properties were not determined from pQCT scans, but rather from histological sections as described above.

Statistical Analysis

All data are presented as mean ± standard error of the mean (SEM). Statistical analyses were performed on body mass, vBMD and all geometric and mechanical properties using Student's t-tests, checking for the effects of exercise in each bone and gender (Sigma Stat 2.0, Jandel Scientific). In groups which failed to exhibit normal distributions or equal variance, Mann-Whitney rank sum tests were performed. A value of $p<0.05$ was considered significant while a p-value between 0.05 and 0.10 was also noted as a trend.

RESULTS

At the beginning of the exercise regimen (Day 0), control and exercise groups in each gender were body weight matched. Body weights were again measured at the end of the study (Day 23), with no significant difference noted between exercise and control groups in either gender (Figure 1). Though not significant, both control and exercise male mice gained weight during the study (5.9% in control, 7.4% in exercise). A similar non-significant trend was noted in female control mice (5.6%), but female exercise mice lost weight during the study (−1.2%).

A relatively brief 21 day period of running initiated at 8 weeks of age induced changes in geometric and mechanical properties that most strongly impacted the male tibiae (Figures 2–5). Tibial length was not impacted by exercise in male mice (Male Control: 17.59 ± 0.18 mm, Male Exercise: 17.47 ± 0.12 mm). Exercise significantly increased cortical area (Figure 2A) and medial-lateral (ML) width (Figure 2B) in the tibial mid-diaphysis of males, resulting in a significant increase in bending moment of inertia about the AP axis (Figure 2C). These changes in geometric properties were primarily the result of an exercise-induced increase in periosteal perimeter (indicating greater periosteal formation with exercise, Figure 3A), though endocortical perimeter was also greater (indicating endocortical resorption with exercise, Figure 3B). Volumetric bone mineral density (vBMD) was also significantly increased with exercise (Male Control: 1015 ± 15 mg/cm^3, Male Exercise: 1219 ± 9 mg/cm^3; $p < 0.001$).

At the structural level, there was a significant increase in post-yield deformation in the male tibiae (Figure 4A); however, this occurred at the expense of yield deformation (Figure 4A) and yield force (Figure 4B). Further, there was a marginal decrease in work-to-yield (Figure 4C, $p<0.066$). Similar trends were noted at the tissue level, where strain-to-failure was significantly greater with exercise (Figure 5A), but at the expense of decreased yield and ultimate stress (Figure 5B). Further, tissue-level stiffness was marginally decreased (Figure 5C, $p<0.061$). No exercise-induced effects were noted in any properties in the male femora versus controls (Tables 1–3), with the exception of a decrease in vBMD (Male Control: 1240 ± 5 mg/cm^3, Male Exercise: 1205 ± 6 mg/cm^3; $p < 0.009$). In males, the percent change with exercise in every property except total deformation and work-to-failure was greater in the tibiae than in the femur, indicating a bone specific response to exercise in males.

The bones of female mice exhibited little geometric or mechanical response to the exercise regime (Figures 3–5, Tables 1–3). No geometric (Table 1) or structural mechanical properties (Table 2) were impacted in the femora, though structural stiffness was marginally decreased with exercise ($p<0.072$). At the tissue-level, modulus of elasticity was significantly decreased (Table 3), while ultimate stress was marginally decreased ($p<0.082$). Tibial length was not impacted by exercise in female mice (Female Control: 17.13 ± 0.19 mm, Female Exercise: 17.16 ± 0.19 mm), nor was any other tibial property (Figures 2, 4–5). In contrast to males, where a majority of properties in the tibia had a greater response to exercise than in the femur, the female femora and tibia both showed similar lack of response to exercise.

DISCUSSION

This study demonstrates that in growing B6;129 mice, the skeletal response to three weeks of running on a treadmill at a low incline and moderate intensity is bone and gender specific. This short term exercise regime most significantly affected the male tibial diaphysis, which responded to exercise via increased cross-sectional dimensions (Figure 2). Measures of periosteal and endocortical perimeters (Figure 3) reveal that changes in cross-sectional geometry occurred primarily due to periosteal expansion, though endocortical resorption also occurred. While the tibiae from exercise mice had superior structural post-yield deformation and tissue strain-to-failure compared to non-exercise controls, these benefits came at the

expense of reduced yield deformation, structural and tissue-level yield strength, and tissue-level ultimate strength (Figures 4 and 5). Growth is actively occurring in 8 week old mice [32,33]. This study demonstrates that exercise accelerated this growth in the tibiae of male mice by increasing cross-sectional dimensions compared with non-exercise control mice (Figures 2 and 3). Because a baseline group was not included, it is unclear how much bone was forming in the control mice. However, it is clear that due to growth effects, the exercise bones contain more tissue that formed under exposure to mechanical stimulation than the simple difference in size between exercise and control mice. This mechanical stimulation had a profound effect on the forming bone resulting in the observed improvements in post-yield properties.

Because the contribution of bone material to structural strength increases as the square of the distance from the centroid, a large moment of inertia is favorable and normally results in greater structural strength. In this study, however, the loaded tibial diaphyses had more material and a greater moment of inertia, but decreased structural yield strength and yield deformation. Decreases in structural mechanical properties in response to exercise have also been observed in growing rats. For example, ten weeks of strenuous running in 8 week old rats detrimentally affected structural properties of the growing tibiae (decreased yield force and ultimate force, decreased work-to-failure) [34]. Similarly, ten weeks of high intensity running in 4 month old rats decreased twist angle and energy absorption in the femur [21], while three weeks of moderate intensity running in 3 month old rats decreased stiffness but increased twist angle of the tibia [35]. However, to the best of our knowledge, detrimental functional effects in response to moderate exercise or loading have not been demonstrated in mice.

Increased quantity of tissue resulting in decreased structural yield strength implies that changes in tissue quality are driving the decreases in structural properties. While not measured in the current study, it seems likely that exercise-induced changes within the newly forming and pre-existing bone matrix are occurring [36]. The organic matrix, predominantly Type I collagen, dictates post-yield behavior in bone [37–40]. Therefore, it is reasonable to hypothesize that the post-yield changes noted here are a toughening-mechanism in the bone due to alterations in collagen, possibly in the orientation of newly-forming fibers or in the number, quality or maturity of cross-linking as has been shown in other exercise studies using Raman Spectroscopy [41]. However, the integrity of the collagen network and bone strength are not mutually exclusive, since collagen forms the scaffold for mineralization. Differences in collagen that improve post-yield properties could induce alterations in mineral crystal size and/or orientation, resulting in greater vBMD in the exercise bones, but leading to the decrease in tissue-level strength [37,40,42]. It is also possible that given more time between the termination of exercise and sacrifice, newly formed bone may mature and alterations that occurred in the pre-existing bone may further change leading to increased strength [43].

In males, the contrast between a strong response to exercise in the tibia with little response noted in the femora supports the hypothesis of a bone-specific response to running. Others have demonstrated surface or site-specific responses to exercise in a single location in one bone (increased periosteal bone formation with no effect on endocortical formation of the femoral diaphysis; [13,16,44]), two locations in the same bone (greater increase in cancellous bone mass of the distal tibia than in the proximal tibia; [45]) and among different bones [13,20,21, 35]. One problem with many studies investigating the response of bone to exercise is that one bone (e.g. the femur) is used to determine some properties while another bone (e.g. the tibia) is used to determine other properties, making it difficult to detect a differential response in the long bones of the leg. Because the femoral diaphysis is the standard site for investigations in murine cortical bone, the tibial diaphysis is frequently not evaluated. However, especially in murine running models, the tibia is also an important location to investigate due to the proximity of this bone to the point of impact and the orientation of the bones during running. One possible

explanation for the bone specific response to exercise relates to the biomechanics of mice running on a treadmill. We observed that mice tend to run with both hind legs flared out laterally. This qualitatively appears to subject the tibia to a more complex loading state (axial loads, bending and torsion) versus the mostly bending loads on the femora. Perhaps this loading state causes the bone formation threshold to be exceeded in the tibia more easily than in the femora.

Gender specificity in response to exercise in rodents is not unknown [17,18,46,47], but the mechanisms remain elusive and are most often attributed to hormonal differences between the genders. The tibiae of young female mice can respond to loading [48–52] and exercise [19]. The fact that this tibial response to loading and exercise is regulated by genetic factors [19, 48,51] may explain why mice from the hybrid strain used in this study were unable to respond. The hybrid strain was used because mice from this strain have been utilized as the wild type background strain for many genetic knockouts [25,53,54]. All mice in the current study were wild type animals which have a low bone mass phenotype that is generally similar to that of C57BL6 mice [55].

At baseline, the mean body weight of female mice was less than males (Figure 1), whereas cortical bone area and bending moment of inertia were slightly larger than in males (NS, Figure 2). Even with running, a critical strain threshold for stimulating increases in bone formation activity [56,57] may never have been surpassed in females suggesting that a longer duration or greater intensity of running might be necessary in female mice [17,19,58]. The actual strains experienced by each bone during running could be determined directly using strain gauges, or indirectly using a finite element model. However, these experiments are beyond the scope of the current study, and strain-gauging mice during running is non-trivial.

Differences in growth rates between male and female mice may be contributing to the disparity in response to mechanical loading [47]. One of the limitations of the current study is that formation activity using fluorchrome labeling was not performed. A further limitation is that a baseline group of mice was not included. Therefore, it is not possible to determine how the effects of growth and growth rate during exercise are influencing the properties that are measured.

Unlike mice in voluntary running studies, the mice in the current study were forced to run on a treadmill. This speed and daily duration were considered moderate and were sufficient to elicit a bone formation response in male (5 weeks old) and female (7 weeks old) mice [16, 59]. However, the 3 week duration of the exercise regimen used in the current study is 1 week shorter than in these previous studies and at a lesser incline in an attempt to elicit a response in the shortest time possible. With this type of forced exercise, it is possible that the exercise mice were subjected to an increased stress response which has implications to bone health. Though not measured in the current study, the stress effects associate with running on a treadmill have been investigated (data not shown). Hormone levels (testosterone, corticosterone) were measured at various time points in the blood, and body weight was tracked every day. At the end of the study, geometric and mechanical properties were measured. No discernible effects on hormone levels were found with exercise or stress. Geometric and mechanical properties in the stress group were the same as in the control group, suggesting that the effects of exercise on these properties were truly the results of mechanical loading and not stress.

In summary, this study demonstrates that in growing B6;129 mice, the response to three weeks of running on a treadmill is bone and gender-specific. As opposed to the minimal effect noted in females and in the male femora, this short-term exercise regime impacted the male tibial diaphysis, which responded to exercise via an increase in periosteal perimeter, endocortical

perimeter, tissue area and distribution compared with growth alone. The exercise mice had greater structural post-yield deformation and tissue-level strain, but these benefits of exercise came at the expense of tissue-level strength, and structural yield strength and deformation. These results suggest that exercise superimposed upon growth accelerated growth-related increases in tibial cross-sectional dimensions. Exercise also influenced the quality of this forming bone, significantly impacting structural and tissue-level mechanical properties.

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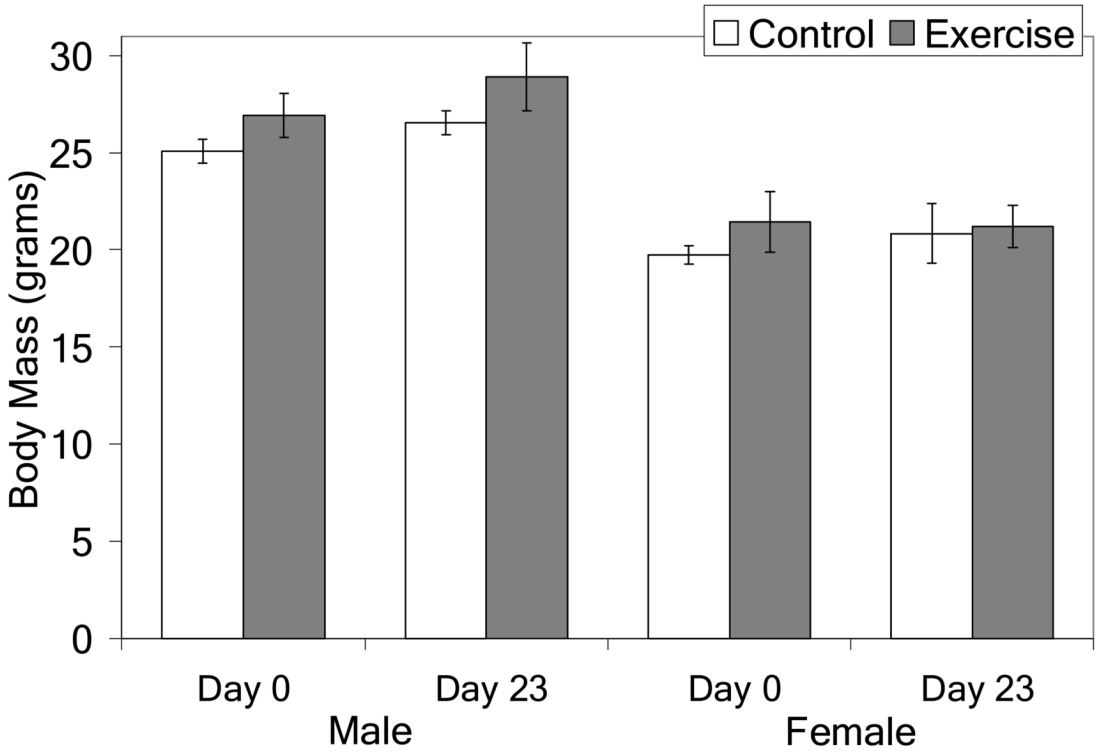

Figure 1. Body Weights of Male and Female C57BL6/129 Mice Prior To and Following Exercise
At the beginning of the study at 8 weeks of age (Day 0), control and exercise groups in each gender were body weight matched. Body weights were again measured following sacrifice (Day 23), with no difference noted between exercise and control groups in either gender. Data are presented as mean ± SEM.

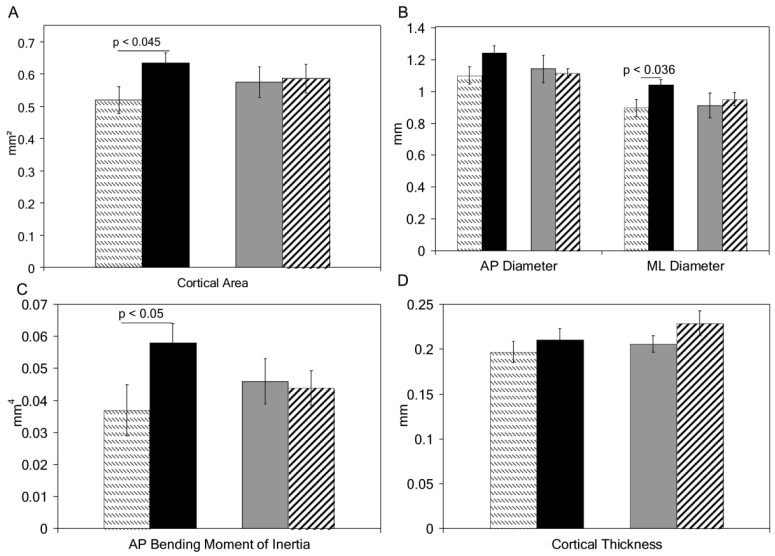

Figure 2. Geometric Properties of the Male and Female Tibial Diaphyses Following Exercise

In C57BL6/129 males, 3 weeks of exercise beginning at 8 weeks of age resulted in a statistically significant increase in cortical area (A) and medial-lateral (ML) width (B) at the tibial mid-diaphysis, resulting in an increase in Anterior-Posterior (AP) bending moment of inertia (C). No changes were noted in any properties in females. Data are presented as mean ± SEM. ▨ = control male, ■ = exercise male, ▩ = control female, ▨ = exercise female.

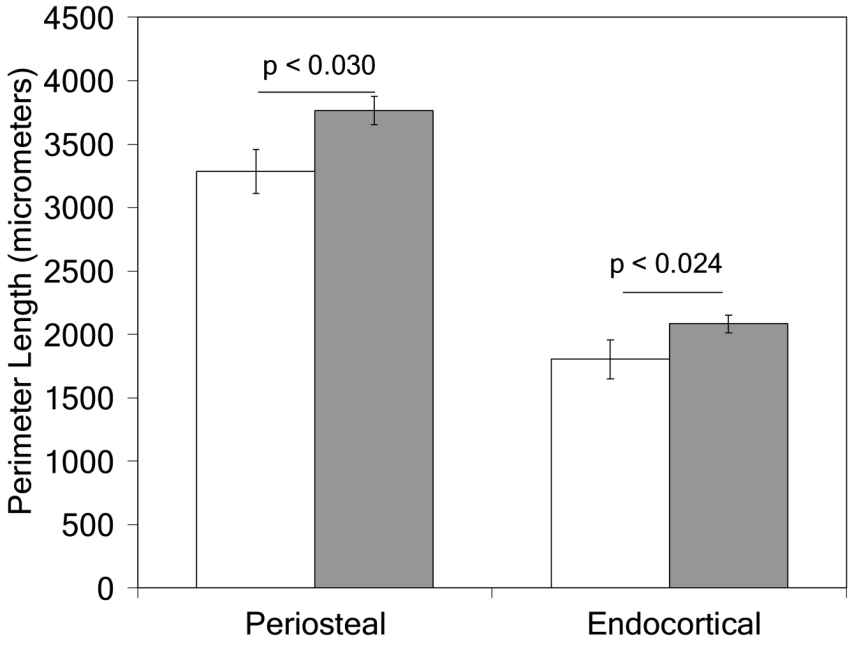

Figure 3. Periosteal and Endocortical Perimeters of the Male Tibial Diaphyses Following Exercise
In C57BL6/129 males, 3 weeks of exercise beginning at 8 weeks of age resulted in a statistically significant increase in periosteal and endocortical perimeter, indicating greater periosteal formation and endocortical resorption in the exercise mice. Data are presented as mean ± SEM. □ = control male, ■ = exercise male

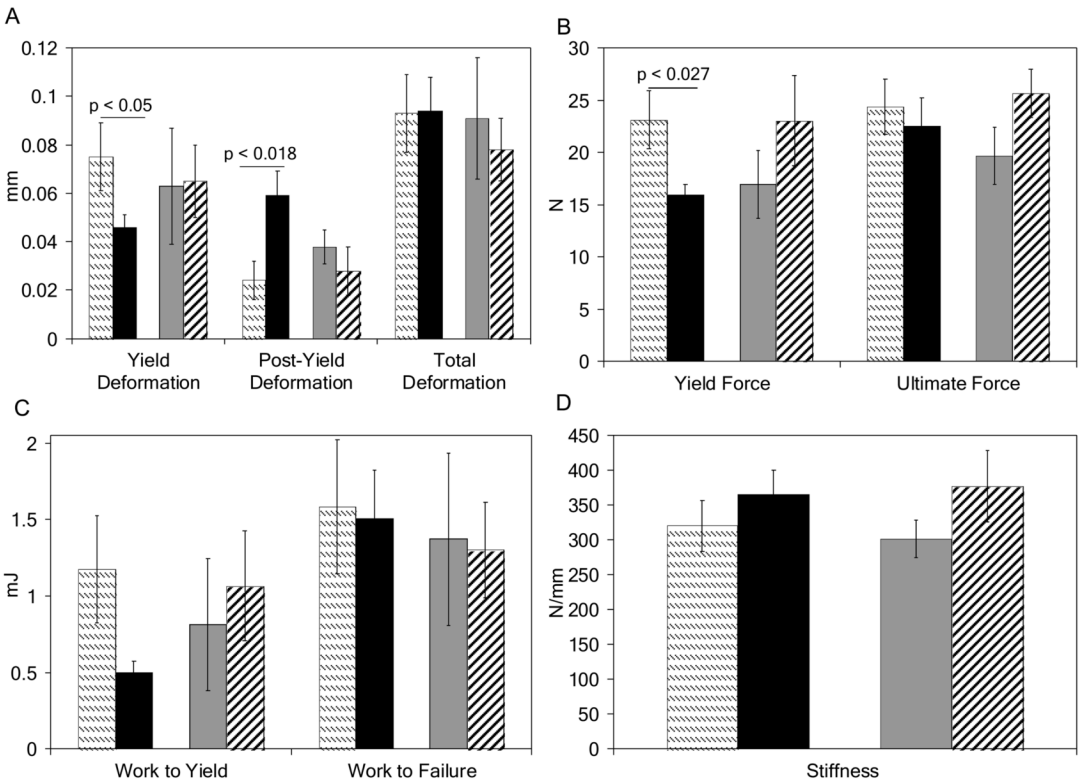

Figure 4. Structural Level Mechanical Properties of the Male and Female Tibial Diaphyses Following Exercise

In C57BL6/129 males, 3 weeks of exercise beginning at 8 weeks of age increased tibial post-yield deformation (A), but at the expense of yield deformation (A) and yield force (B). No changes were noted in any properties in females. Data are presented as mean ± SEM. ▨ = control male, ■ = exercise male, ▩ = control female, ▨ = exercise female.

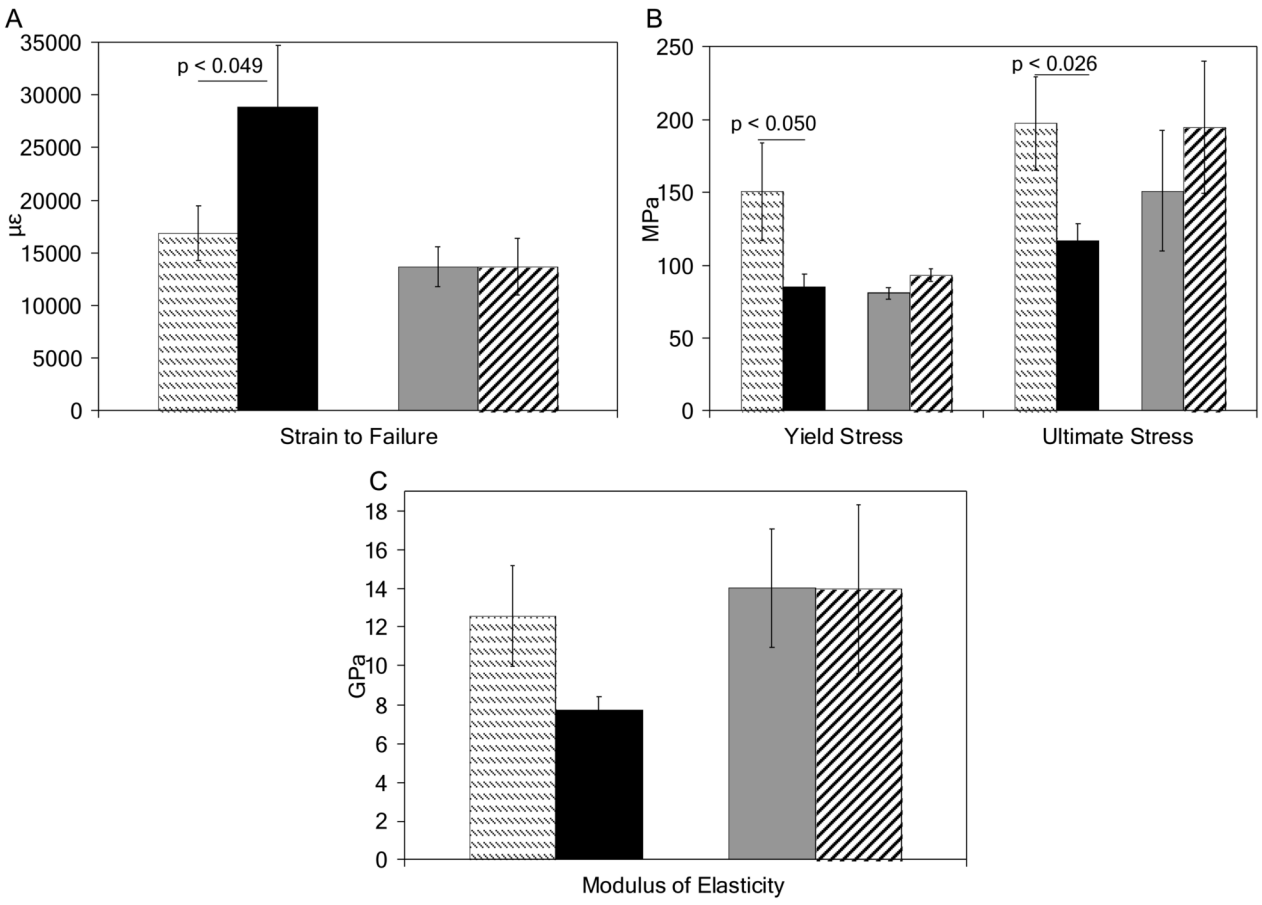

Figure 5. Tissue Level Mechanical Properties of the Male and Female Tibial Diaphyses Following Exercise

In C57BL6/129 males, 3 weeks of exercise beginning at 8 weeks of age increased tibial strain to failure (A), but at the expense of yield and ultimate tissue strength (B). No changes were noted in any properties in females. Data are presented as mean ± SEM. ▨ = control male, ■ = exercise male, ▩ = control female, ▨ = exercise female.

Table 1

Geometric Properties of the Male and Female Femoral Diaphyses of Control and Exercise Mice

Group	Length (mm)	Cortical Area (mm)	AP Diameter (mm)	ML Diameter (mm)	Moment of Inertia (mm^4)	Average Thickness (mm)
Male Femora (Control)	13.94 ± 0.82	0.88 ± 0.05	1.16 ± 0.02	1.78 ±0.12	0.103 ± 0.003	0.224 ±0.013
Male Femora (Exercise)	14.71 ± 0.04	0.85 ± 0.07	1.18 ± 0.04	1.69 ± 0.06	0.113 ± 0.013	0.223 ± 0.019
Female Femora (Control)	14.42 ± 0.07	0.80 ± 0.03	1.23 ± 0.02	1.63 ± 0.04	0.110 ± 0.007	0.224 ± 0.018
Female Femora (Exercise)	14.22 ± 0.28	0.76 ± 0.02	1.19 ± 0.03	1.64 ± 0.09	0.115 ± 0.013	0.241 ± 0.021

Geometric properties were determined from histology at the fracture site. Data are presented as mean ± SEM.

Table 2

Structural Mechanical Properties of the Male and Female Femoral Diaphyses of Control and Exercise Mice

Group	Yield Force (N)	Ultimate Force (N)	Yield Deformation (mm)	Post-Yield Deformation (mm)	Total Deformation (mm)	Stiffness (N/mm)	Work to Yield (mJ)	Work to Failure (mJ)
Male Femora (Control)	21.38 ± 2.68	29.06 ± 2.89	0.080 ± 0.015	0.061 ± 0.017	0.141 ± 0.024	274.3 ± 41.8	1.06 ± 0.21	2.63 ± 0.54
Male Femora (Exercise)	21.33 ± 2.56	29.34 ± 1.59	0.080 ± 0.008	0.074 ± 0.026	0.153 ± 0.020	265.5 ± 24.1	1.04 ± 0.19	2.86 ± 0.45
Female Femora (Control)	26.01 ± 1.66	35.91 ± 1.59	0.066 ± 0.012	0.054 ± 0.008	0.120 ± 0.014	**495.6 ± 38.0**	1.02 ± 0.14	2.75 ± 0.32
Female Femora (Exercise)	26.76 ± 3.35	33.49 ± 2.85	0.084 ± 0.019	0.037 ± 0.01	0.115 ± 0.01	**364.2 ± 49.3**[b]	1.42 ± 0.35	2.50 ± 0.43

Data are presented as mean ± SEM.

[b] indicates 0.05<p<0.10 vs. control for the same gender.

Table 3

Tissue-Level Mechanical Properties of the Male and Female Femoral Diaphyses of Control and Exercise Bones

Group	Yield Stress (MPa)	Ultimate Stress (MPa)	Strain to Failure (με)	Modulus of Elasticity (GPa)
Male Femora (Control)	62.75 ± 10.10	85.05 ± 11.64	36000 ± 5446	3.13 ± 0.50
Male Femora (Exercise)	56.67 ± 7.05	78.17 ± 5.33	38637 ± 5021	2.78 ± 0.23
Female Femora (Control)	73.47 ± 4.03	**101.49 ± 3.00**	31804 ± 4617	**5.26 ± 0.19**
Female Femora (Exercise)	74.67 ± 11.44	**90.23 ± 6.83**[b]	29878 ± 3236	**3.73 ± 0.40**[a]

Data are presented as mean ± SEM.

[a] indicates $p < 0.05$ vs. control for the same gender.

[b] indicates $0.05<p<0.10$ vs. control for the same gender.